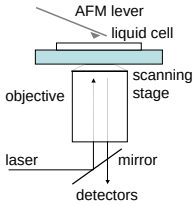
Scanning Probe and Fluorescence Microscopy of the Nano-Bio Interface

"Thought is impossible without an image" Aristotle, 325 B.C.

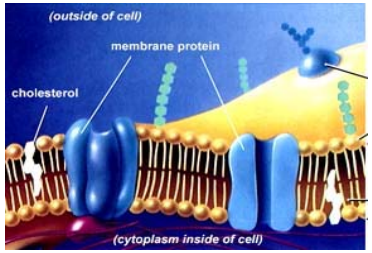
Imaging the nano-bio interface

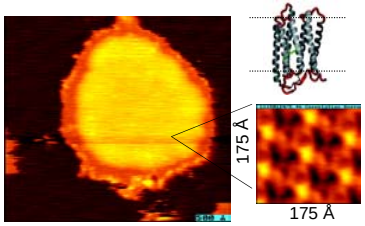
- Advances in the understanding of biomolecular function and integration will be aided by molecular-level imaging capabilities that provide:
  - High lateral (nm) and vertical resolution (<1nm)
  - Single-molecule detection sensitivity
  - Fluid environment
- Here we highlight the current imaging capabilities based on atomic force microscopy (AFM) and confocal fluorescence imaging.
- As shown below, we can also combine both into a single instrument for simultaneous imaging.



Membrane studies

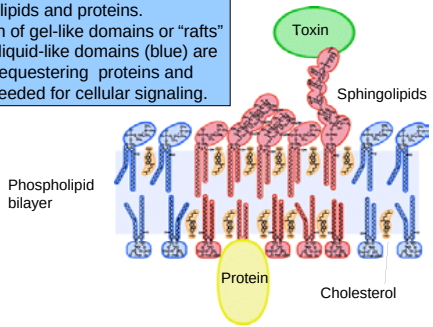
- The cellular membrane is the ultimate biomolecular interface for molecular detection, transport, etc.
- In molecular-level studies of both model membranes and biological membranes we can begin to understand how lipid and protein components of the membrane are structurally organized for optimum function.



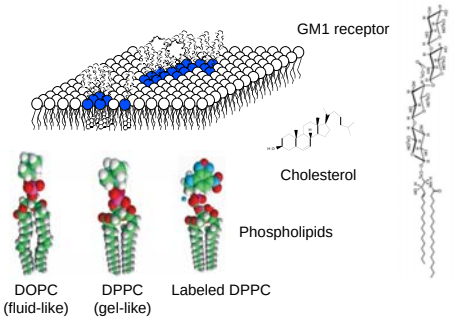


High resolution imaging of the "Purple membrane" *(Halobacterium salininarum)* on mica reveals the hexagonal array of the light-harvesting trans-membrane protein bacteriorhodopsin.

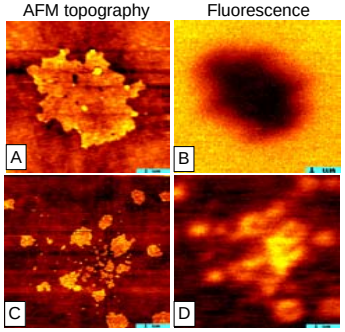
• Membranes are multi-component mixtures of lipids and proteins.
• Separation of gel-like domains or "rafts" (pink) from liquid-like domains (blue) are critical for sequestering proteins and receptors needed for cellular signaling.

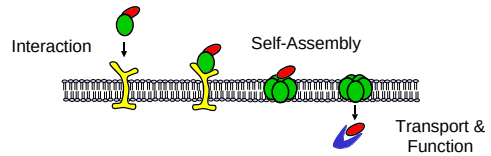


Model studies of membrane organization are underway. We are using various lipids shown below to study the factors that give rise to lipid "rafts." These include lipid tail structure, head group interactions, sterol packing, and toxin-receptor interactions.

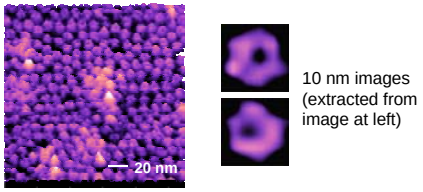


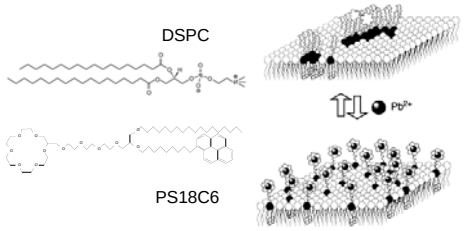
Simultaneous AFM and fluorescence images of model membranes are shown below. In (A) DPPC rafts are 1 nm higher than the surrounding DOPC. In (B), it can be seen that a head-labeled fluorescent DPPC probe is unexpectedly excluded from the raft. In (C) and (D), the DPPC rafts include GM1 receptor which binds fluorescent cholera toxin. (Scale bars = 1μm)



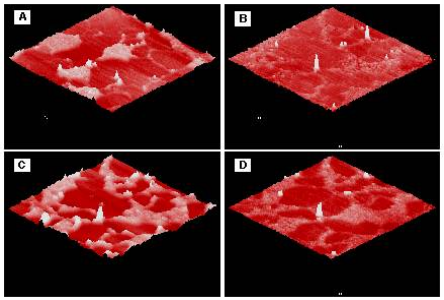


We are using AFM and model membranes to study the mechanisms that AB toxins use to breach cell membranes. Above we depict the initial binding of toxin B domain (green) to a receptor, followed by oligimerization and pore formation. The toxin A domain (red) then translocates into the cell. Below we show AFM images of pentamers of cholera toxin B domains bound to GM1 receptor sites.





AFM is used to follow the dynamic re-organization of PS18C6 receptor sites upon binding of charged Pb^{2+} ions. The matrix lipid is DSPC (above). Images below demonstrate the electrostatic actuation of the film from aggregated to dispersed states with Pb^{2+} ions. The membrane in the (A) initial state, then (B) after addition of 0.1 mM $Pb(NO_3)_2$, followed by (C) addition of 0.1 mM EDTA to remove metal ions from the membrane, and finally (D) after addition of 0.1 mM $Pb(NO_3)_2$ again.



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